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Novel mustard meal extract for control of the potato cyst nematode, *Globodera pallida*

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Plant parasitic nematodes are among the most invasive and widespread pests of crops. Difficult to detect and control, nematode problems are insidious and can be costly. Often less obvious than symptoms caused by other pests or pathogens, symptoms of nematode infection are not specific and are often mistaken for drought or nutrient stress. Plants are rarely killed outright, but rather patches of plants in a field may appear stunted, wilted, or yellow and damage can be manifested gradually over many years. If left untreated, nematode attack can substantially reduce yield. In the United States, nematode-related damage has been estimated to reduce yield by nearly 25% (Koening and Overstreet, 1999). Globally, nematode damage is estimated to reduce yield by 12% at a cost of $157 billion (Sing et al, 2015).

In potato production, two nematode species top the list in importance. The invasive and highly damaging potato cyst nematodes, *Globodera rostochiensis* and *Globodera pallida*, are internationally recognized quarantine pests. In highly infested fields, potato cyst nematodes can reduce yields up to 80%. Although not widely distributed in the United States, both are present and are regulated by USDA-APHIS and the relevant state agency, either the Idaho State Department of Agriculture (ISDA) or the New York State Department of Agriculture (NYSDA). The golden nematode, *G. rostochiensis*, first discovered in New York, has been contained to 5,945 acres in eight counties. The pale cyst nematode (PCN), *G. pallida*, infests 3,277 acres in portions of two counties in Idaho. Spread mainly through soil contaminated with cysts (Fig. 1), adherence to a stringent phytosanitary program has

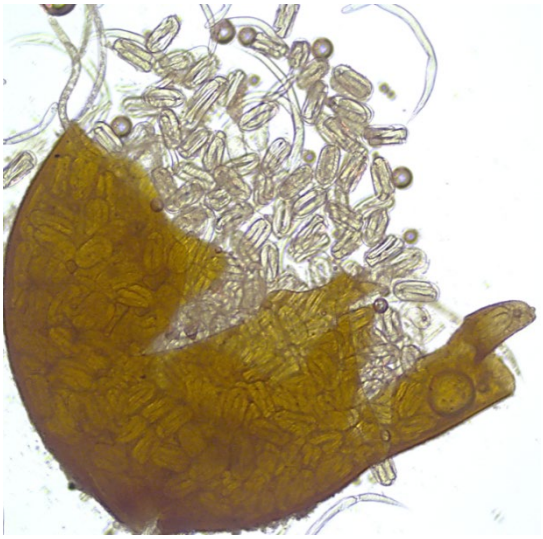

Figure 1. *Globodera pallida* cyst, filled with unhatched eggs.

prevented spread of cysts to other potato-growing areas; PCN remains contained to less than 1% of the total acreage planted to potato in Idaho.

For the United States, *G. pallida* is a quarantine pest for which there is zero tolerance. The presence of *G. pallida* in Idaho was viewed with extreme alarm by other states and countries that import Idaho potatoes. Prior to implementation of the current quarantine measures, import of Idaho fresh potato products and nursery stock was banned by some of Idaho's most important trading partners including Canada, Mexico, and Japan. Consequently, eradication of *G. pallida* is a top priority for the Idaho potato industry. A critical component of this work has been undertaken by USDA-APHIS through fumigation of infested fields with methyl bromide (MeBr). However, MeBr use was discontinued in 2015, and in 2016 the registrant pulled their supplemental Idaho label. USDA-APHIS currently relies on fumigation with 1,3-dichloropropene.

Despite the presence of resistant varieties, it is estimated that 9% of the total annual potato crop in the United Kingdom, or $70 million, is lost because of potato cyst nematodes. In Idaho, where potato cannot be planted on infested land, preliminary data suggest that PCN costs the state approximately $25 million per year. Given the current withdrawal from use of methyl bromide and the very limited availability of other inorganic nematicides, the need to consider other strategies for nematode control has become acute. Potentially viable alternatives exist in the form of natural products derived from plants in the mustard family.

Plants in the mustard family produce a broad range of glucosinolates (GSLs) that hydrolyze to toxic metabolites that can be utilized in biofumigation for nematode control. Sinigrin, which is found in high levels in *Brassica juncea* or Oriental mustard, was identified as the GSL with the most activity. We have shown that sinigrin in *B. juncea* seed meal can eliminate PCN. Exposure to *B. juncea* seed meal when applied at a rate of 4 t/ac, completely killed PCN, but decreasing the application rate to a more feasible amount (2.0 t/ac) resulted in variable kill of PCN (Table 1). The rate of mustard meal required for field application limits the utility of using meals in nematode-control strategies. To overcome this obstacle, we have developed procedures to extract the active ingredient (sinigrin) from mustard seed meal, concentrate the extracts, and formulate shelf-stable powdered products (Fig. 2).

Table 1. Percentage hatch of *Globodera pallida* eggs exposed to *Brassica juncea* seed meal extract (2 ton/acre) in Shelly, ID.

Treatment	Hatch (%)
Bare soil	19.0 ± 2.9
B. juncea extract	1.9 ± 2.8
P-value	*0.001*

[y]Values ± standard errors are the average of six replicates.

Figure 2. Mustard seed meal extract in a wettable powder form.

Our shelf-stable sinigrin extract effectively kills PCN even at low rates of application. In our Shelley Idaho field trial, exposure of PCN to *B. juncea* seed meal extract containing the active ingredient sinigrin reduced egg hatch by 90% compared to a non-amended control (Table 1). In greenhouse experiments with PCN, *B. juncea* seed meal extract significantly reduced hatch by 97.0 to 99.7% at all rates (0.5 – 2 tons/acre)

tested compared to the non-amended control (Table 2). Subsequent to biofumigation, PCN was unable to reproduce on potato when extract was applied at 1 ton/acre, whereas reproduction was reduced by 99% when applied at 0.5 ton/acre (Table 2). The ability to reduce the amount of material being applied to soil by using an extract has the potential for integration into a *G. pallida* eradication program.

Table 2. Percentage hatch of *Globodera pallida* eggs after 2 weeks exposure to five rates of *Brassica juncea* seed meal extract, followed by a greenhouse bioassay to assess reproduction of exposed cysts on potato.

Extract rate (tons/acre)	Hatch (%)	Cysts/pot
Non-amended	9.6 ± 4.0 a	60.4 ± 26.8 a
0.5	0.8 ± 0.8 b	0.2 ± 0.2 b
1.0	0.6 ± 1.3 b	0 b
1.5	0.4 ± 0.5 b	0 b
2.0	0.2 ± 0.4 b	0 b

[y] Values ± standard errors are the average of six replicates. Values within a column followed by a common letter are not significantly different according to Tukey-Kramer test ($P < 0.05$).

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